THE ACTION OF THE BACILLUS PESTIS ON CERTAIN CARBO-HYDRATES AND ALLIED BODIES IN LIQUID MEDIA; AND ON THE ADVANTAGE OR DISADVANTAGE OF EMPLOYING SUCH MEDIA FOR THE MANUFACTURE OF AN ANTI-PLAGUE VACCINE.

By W.B.Bannerman,M.D.,B.Sc.,Lt-Colonel,I.M.S.,
Director,Bombay Bacteriological Laboratory,
Parel, Bombay, India.

In a former paper, the production of alkali in liquid media by the Bacillus pestis was described by me,(§) and it is the purpose of the present paper to discuss the action of the same organism on the various carbo-hydrates and allied bodies, which have been used by bacteriologists for the differentiation of various species of intestinal and other bacteria.

As the investigation proceeded it was found that the action of the bacillus differed according to the carbo-hydrate or other body employed, and that these therefore fell into two distinct groups. In the one group it was found that an alkaline reaction resulted from the growth of the plague bacillus, while in the other,- on the contrary,- an acid reaction was found to be present when the medium was tested after an equal period of time.

In the paper above alluded to, it was

(§)The Production of Alkali in Liquid Media by the Bacillus pestis. By Lieut-Colonel W.B.Bannerman,M.D. B.Sc.,I.M.S. Scientific Memoirs by the officers of the Medical and Sanitary Depts.Gt.of India. No.33.

demonstrated that the plague bacillus, when grown in the ordinary broth medium of the laboratory, produced an alkali, which alkali was the cause of the cessation of growth of the B.pestis. From a consideration of the results obtained by Sir A.E.Wright and his fellow-workers we may assume that a bacterial vaccine,- such as the plague prophylactic of Haffkine, which is produced by the growth in broth for a number of weeks of the B.pestis,- is efficient in proportion to the number of bacilli in a given bulk of fluid.

From the point of view of the Bombay Bacteriological Laboratory, in which is prepared the whole of the enormous quantity of anti-plague vaccine used in India, and which is responsible for its purity, it seemed important to find out whether the addition of any of these carbo-hydrates or allied bodies would be useful in prolonging the period of activity of the plague bacillus, and so perchance favouring a more vigorous and thicker growth in the medium.

For it seemed quite possible that some of these bodies which did not permit of the formation of alkali by the plague bacillus might favour its growth in some way. This matter was investigated by me during the course of the many months the various cultures were under observation. In addition, some experiments on animals were made to ascertain if a vaccine made from any particular medium was superior in efficacy to any other.

These animal experiments are described in

some detail at the end of this paper, and the results summarised.

That the plague bacillus has the power of acting on some of the carbo-hydrates and the allied bodies, with the resulting production of acid has been known for some time. For instance Macé says, "Le microbe produit un peu d'acide dans les milieux, probablement aux dépens des hydrocarbones".(§) Again MacConkey (1905) in a paper on the lactose-fermenting bacteria in faeces reports (:) that he found that glucose, laevulose, maltose, galactose, mannite and dextrin, acid was produced by the action of B.pestis, while in the case of raffinose, lactose, cane-sugar, sorbite and dulcite, no such action was produced. The second Plague Commission of the Government of India repeated some of these experiments and confirmed them so far as regards glucose, lactose, dulcite, laevulose, mannite and galactose.(@)

Up to the publication last year of my paper above alluded to, no one however seems to have known that in ordinary media the B.pestis produced

---

(§) Traite Pratique de Bacteriologie, par E.Macé, Cinquieme Edition, Paris: Libraire J.B.Balliere et Fils, 1904.

(:) Lactose-fermenting Bacteria in Faeces. By Alfred MacConkey, M.B., D.P.H. Journal of Hygiene, Vol.V, No.3, July, 1905, p.350. Cambridge, University Press.

(@) On the differential Diagnosis of the Plague Bacillus from Allied Organisms. Part XXXII, Plague Commission Report Journal of Hygiene Vol.VIII, No.2, 1908.

alkali, nor, that when some of the carbo-hydrates were added to the liquid, a similar phenomenon likewise manifested itself.

The carbo-hydrates used were the following:-

Inulin $(C_6H_{10}O_5)n$

Dextrin $(C_6H_{10}O_5)n$

Raffinose $C_{18}H_{32}O_{16}$

Saccharose $C_{12}H_{22}O_{11}$

Amylum $(C_6H_{10}O_5)n + xH_2O$

Laevulose $C_6H_{12}O_6 = CH_2OH.(CHOH)_3CO.CH_2OH.$

Galactose $C_6H_{12}O_6 = CH_2OH.(CHOH)_4CHO$

Glucose $C_6H_{12}O_6 = CH_2OH.(CHOH)_4CHO$

Maltose $C_{12}H_{22}O_{11}$

Arabinose $C_5H_{10}O_5$

Lactose $C_{12}H_{22}O_{11} + H_2O$

In addition the following alcohols were used:-

Dulcite $C_6H_8(OH)_6$

Sorbite $C_6H_8(OH)_6$

Mannite $C_6H_8(OH)_6 = CH_2OH.(CHOH)_4CH_2OH$

These were added to the ordinary laboratory broth medium in the amount necessary to make a one per cent solution. Tubes with ten c.c. of these various broths were prepared in the ordinary way by steaming for twenty minutes on three days in succession, and the sterility was established by keeping for a week at room temperature, which in the climate of Bombay has been found by experience to be quite sufficient. It may be well at this

point to call attention to the effect of a tropical climate in causing evaporation of liquid media and consequently their increasing concentration as time elapses; it being quite common to find a tube of broth with its contents lessened by a fifth or a quarter at the end of six weeks. The effect of this will be seen in the charts appended, e.g. the increase in acidity shown in the case of the unsown broths.

The method of experiment was the following. Before the various solutions were sown with the plague germ the reaction of each was tested to phenolphthalein. A certain proportion of tubes were then sown and the remainder kept unsown for subsequent testing to serve as controls. The method of carrying out the test has been described in the paper on the production of alkali by the plague bacillus above referred to (p.8) and need not therefore be again detailed. It may suffice to say here that each tube of medium in which the plague germ had been gr growing was itself, before being submitted to the phenolphthalein test, actually tested for purity of growth by taking from it with a sterile pipette a few drops, which were then inseminated on the surface of a dried agar slope. These agar slopes were carefully examined a week afterwards and the state of the resulting growth, or its absence, noted. As a careful examination of plague growth on similar dry agar slopes has been a part of my daily duty for several years past in my capacity as Director of the Bombay Bacteriological Laboratory, which is

responsible for the preparation and purity of Haffkine's plague prophylactic, it is permissible for me to say that a very large and varied and exceptional experience has been thus acquired, and that it is not likely therefore that any serious mistake in the interpretation of the appearances can have been made. In the description of these appearances it is frequently noted that the growth is "Good" or "Very good", which means that a uniform pearly growth was seen covering the entire surface of the agar slope, and this, experience teaches, means that the plague germ is in a vigorous healthy state. In other cases the description runs as follows, "Large colonies only", or "A few discrete colonies only". These appearances denote (§) that the plague germ producing them is in a weak unhealthy state, and it will be noted that very frequently the record for the week following such an entry is "Growth none", proving that the germ which produced only a few scatterred colonies the previous week has now died out altogether, and is no longer capable of growing on an agar medium.

Similarly when in the case of the broth tubes it is noted that there is "No surface growth", it means that the plague germ in that particular tube is moribund or actually dead. A reference to the

---

(§) A very good figure of these curious solitary plague colonies, executed under my supervision, will be found in "The Bombay Plague, being a History of the Progress of Plague in the Bombay Presidency from Sept.1896 to Jan.1899. By Captain J.K.Condon. Bombay., 900

tables will show this, for it will be noted how often the description "A few scatterred colonies only" in the column of "Growth on Agar", is followed in the column descriptive of the condition of the broth tubes by the above sentence. Again it has been my experience to find that when there is no surface growth in broth media, owing to increase of alkali due to the growth of the B.pestis, or to a faulty adjustment of the reaction of the medium to begin with, that then growth may be found along the sides of the tube, having the appearance of small islands or dots. Examples of both these conditions abound in the tables where the results are set forth in full detail, and their meaning must be kept clearly in mind when interpreting the results. These islands of growth on the sides of the containing vessel are frequently the first signs of successful culture when one inseminates a flask of broth with material from a plague bubo, the surface and stalactite growth appearing later. It is also often seen when a plague culture is about to die out.

Having cleared the ground by these preliminary remarks we may proceed to consider in detail the series of experiments which were carried out by me.

Two complete series of experiments with 14 different carbo-hydrates or allied bodies, were made. These chemical substances were procured

direct from E.Merck of Darmstadt, which is sufficient guarantee of their purity. Great care was taken in the sterilisation of the media after they were added to the ordinary laboratory broth, so that no decomposition by over-heating should take place. The colour of the resulting medium was in all cases the same as the broth before the addition of the chemical bodies, with the exception of two, viz.,laevulose and arabinose, where a somewhat darker colour was observed. The reason of this difference is not apparent, for the media containing the different chemicals were all made at the same time and therefore were exposed to the same conditions throughout. A sufficient number of tubes of each medium was made to allow of testing for several weeks in succession after insemination, and a corresponding number were not sown and reserved for testing from time to time as a comparison. In this way changes due to climatic conditions were eliminated as far as possible.

The first experiment was begun in February 1907, the tubes being sown with plague on the 21st.and 22nd.of that month, about a fortnight after the various media were prepared. The start-point of this series is the reaction of the broths as determined immediately before sowing, but unfortunately no observations were made on unsown broths from week to week subsequent to this preliminary trial. The question of gas formation also was not investigated in the case

of this first series, but as this was fully gone into in the second series of experiments the omission is not of any moment. The last observation of this set was made on the 15th.of April, that is, 52 days after the sowing of the plague germ. The second set of experiments began on the 13th.and 14th.of May, and lasted till the 22nd. of June, 1907, a period of 40 days. This was a fuller and better series than the first, as here a parallel series of observations on unsown media was made, and likewise the question of gas formation was decided.

To make the tables of observations clear, charts have been constructed showing graphically the change in reaction of the media brought about by the action of the plague bacillus, and the dates of testing. The figures along the vertical represent the percentage of normal acid present, those along the horizontal the time, in days, that has elapsed after sowing.

The first thing that is apparent from an examination of these charts and tables, is, that in the media containing the first six chemical bodies, namely,-inulin, dulcite, dextrine, raffinose, sorbite and saccharose, alkali is produced by the action of the B.pestis. In the second series of experiments where the curve of the reaction of the unsown medium is shown alongside of that representing the results got from the inseminated tubes, it will be observed that the ends of the two curves are

widely separated. This is not due solely to the drop in acidity caused by the action of the plague bacillus in producing alkali, but is also contributed to by the concentration of the unsown medium resulting from evaporation owing to the climatic conditions of Bombay.

Again it will be noticed that at the end of the period of observation the agar growths inseminated from these tubes are vigorous and cover the entire surface of the agar. From an inspection of the liquid medium also it is evident that growth is vigorously maintained, for there is still to be seen that surface growth which is only apparent when this is taking place, and the abundant deposit recorded proves that the germ had been propagated rapidly and well during the whole period since it was originally sown. This continuance of growth is quite characteristic of the behaviour of the plague bacillus as we know it in the ordinary laboratory medium, and is an additional proof, if such were needed, that the alkali inhibits the growth of the bacillus, but at the same time does not kill it out. This has been fully set forth in the previous paper and need not be further elaborated here.

In the second place, if we examine the charts relating to the next seven chemical bodies, viz.- amylum, mannite, laevulose, galactose, glucose,

maltose and arabinose, we find the reaction becomes more acid as time goes on, under the action of the B.pestis. There is seen as a rule a sudden rise in acidity which causes the two lines representing the sown and unsown broths respectively, to diverge at first, whereas afterwards they continue more or less parallel as though now acted on by the same conditions. An explanation of this is to be found in the early stoppage of growth and the subsequent death of the plague bacillus due to the presence of the acid. It is evident that the parallelism is due to this cessation of growth and the subsequent gradual and equal evaporation of the liquid in the tubes. The growth on agar proves this, and we have only to examine the entries under the heading "Growth on Agar" in the tables to see how soon the germ is affected by the presence of the acid and how rapidly it dies out. Again in the broth cultures the absence of surface growth and the scanty deposit in the tubes shows with how little vigour the life of the bacillus has been carried on.

Lastly, it would seem that lactose must stand in a category by itself, for after the first drop in acidity characteristic of a medium in which the plague germ produces alkali, we find that during the third or fourth week acid is produced, causing a rise, followed by a drop to normal or below it.

The germ also does not soon die out in this medium, for it is found alive even after 52 days.

In none of the 14 chemical bodies experimented with was any trace of gas formation noted.

The method used for its detection was the employment of a Durham's tube in each test tube of medium experimented with.

SUMMARY.

(1) The B.pestis produces alkali in some of the above chemical bodies, and acid in others.

(2) In those in which alkali is produced the life of the bacillus is at first vigorous, only gradually ceases, and even after a long period the germ is found still alive. It behaves in fact just as it does in the ordinary fluid media of the laboratory.

(3) In those in which acid is produced the growth of the B.pestis is much less vigorous, it soon ceases, and the germ actually dies out at an early date.

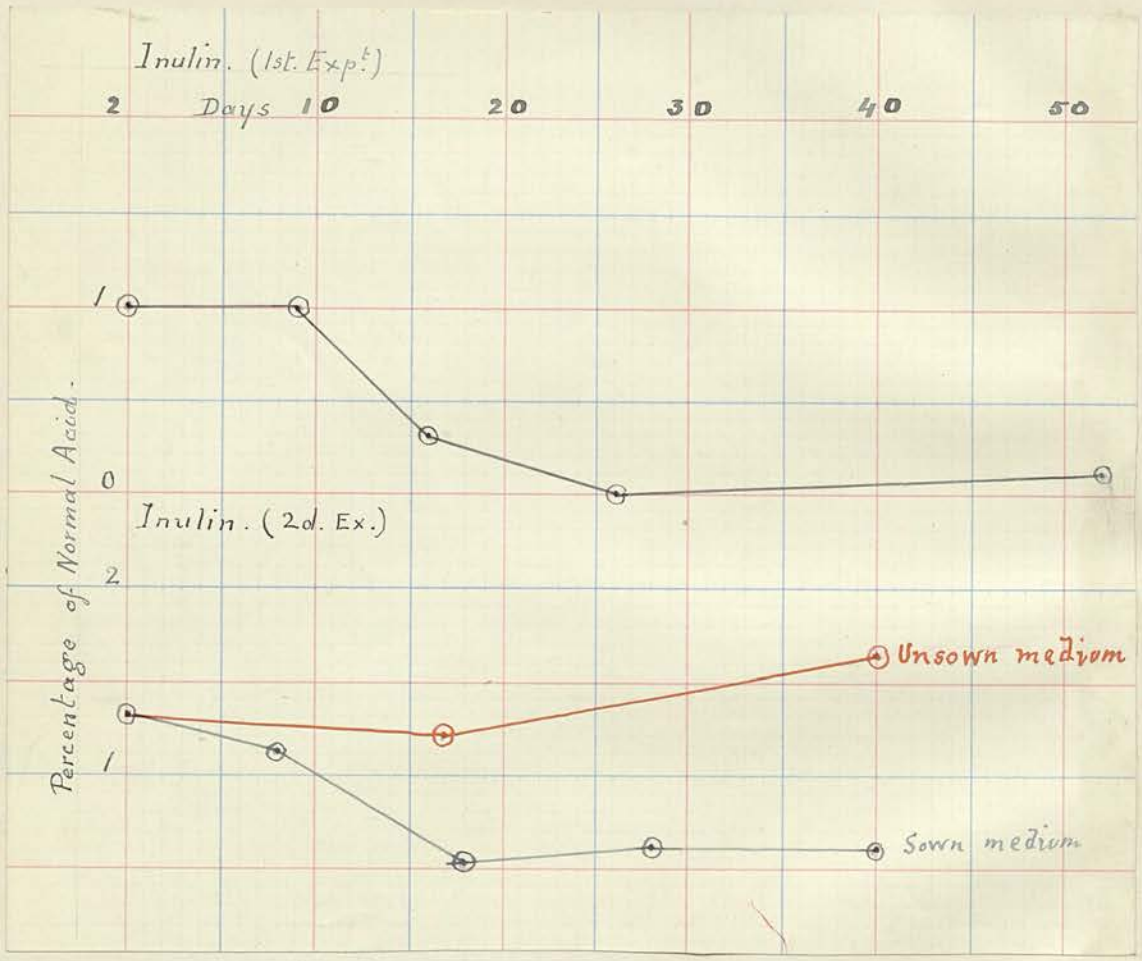
This is exactly what happens when the plague germ is sown in ordinary broth medium made purposely acid.

(4) It seems reasonable to suppose therefore that these chemical bodies as such have no action on the B.pestis, but that the growth or dying out of the germ is due to the formation of alkali in the former case or of acid in the latter.

(5) The formation of gas was not observed in any of the bodies experimented with.

Further experiments, from the chemical point of view, will shortly be carried out to ascertain, if possible, the actual action of the bacillus on the various carbo-hydrates and alcohols, and the nature of the changes brought about in their constitution.

I N U L I N



Good growth on Agar after 40 days in this medium.

FIRST GROUP.

In which the B.Pestis produces alkali.

INULIN. Prepared 5-2-07, sown with plague 22-2-07.

Date	Reaction.	Growth on Agar.	Growth in Broth.
22-2-07	1.0	----	----
2-3-07	1.1	Pure	----
9-3-07	0.3	Pure, abundant.	Abundant surface & heavy deposit.
14-3-07	Neutral	Pure, abundant.	Do. Do. Do.
15-4-07.	0.1	Pure;abundant	Heavy growth.

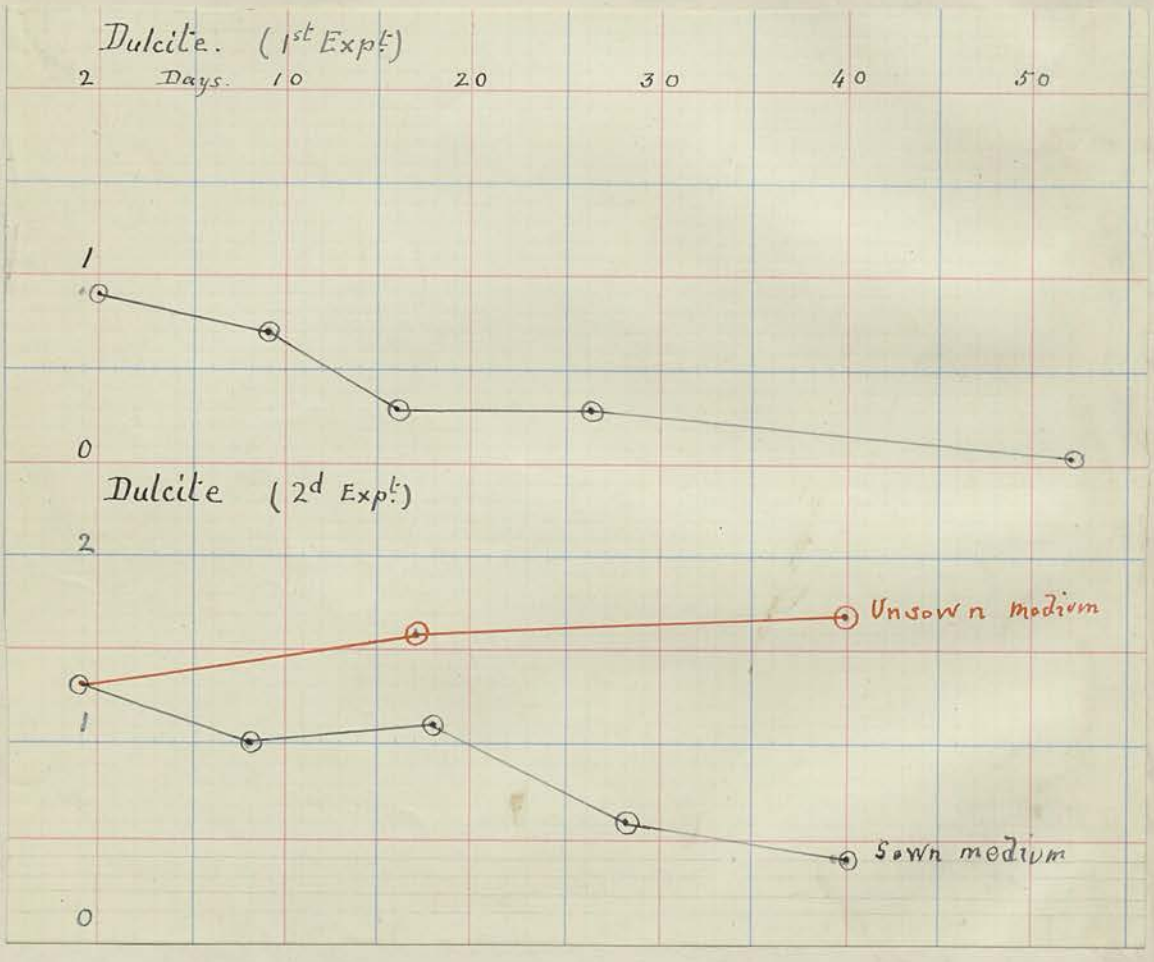
INULIN. Sown with plague 13-5-07.

Date.	Reaction.	Growth on Agar.	Growth in Broth.	Gas formation.
13-5-07	1.3	-----	----	----
22-5-07	1.1	Pure; fair growth.	Some surface growth and along sides	None
1-6-07	0.5	Pure; good do.	Abundant surface do.	None
11-6-07	0.6	Pure; good scattered growth.	Abt. surface growth, & slight along sides. Thick deposit.	None
22-6-07	0.6	Pure; good growth.	Some surface growth, Abundant deposit.	None.

INULIN.broth, not sown with anything.

Date	Reaction	
13-5-07	1.3	Colour of medium like ordinary broth medium.
1-6-07	1.2	
22-6-07	1.7	

D U L C I T E



Good growth on Agar after 40 days in this medium.

FIRST GROUP

(Continued)

DULCITE. Prepared 5-2-07; sown with plague 22-2-07.

Date	Reaction.	Growth on Agar.	Growth in Broth.
22-2-07	0.9	---	---
2-3-07	0.7	Pure	---
9-3-07	0.3	Pure; very abundant.	Abundant surface growth and thick deposit.
19-3-07	0.3	Pure; good growth.	No surface growth, but thick deposit.
15-4-07	0.1	Pure, do. do.	No surface growth, but thick deposit.

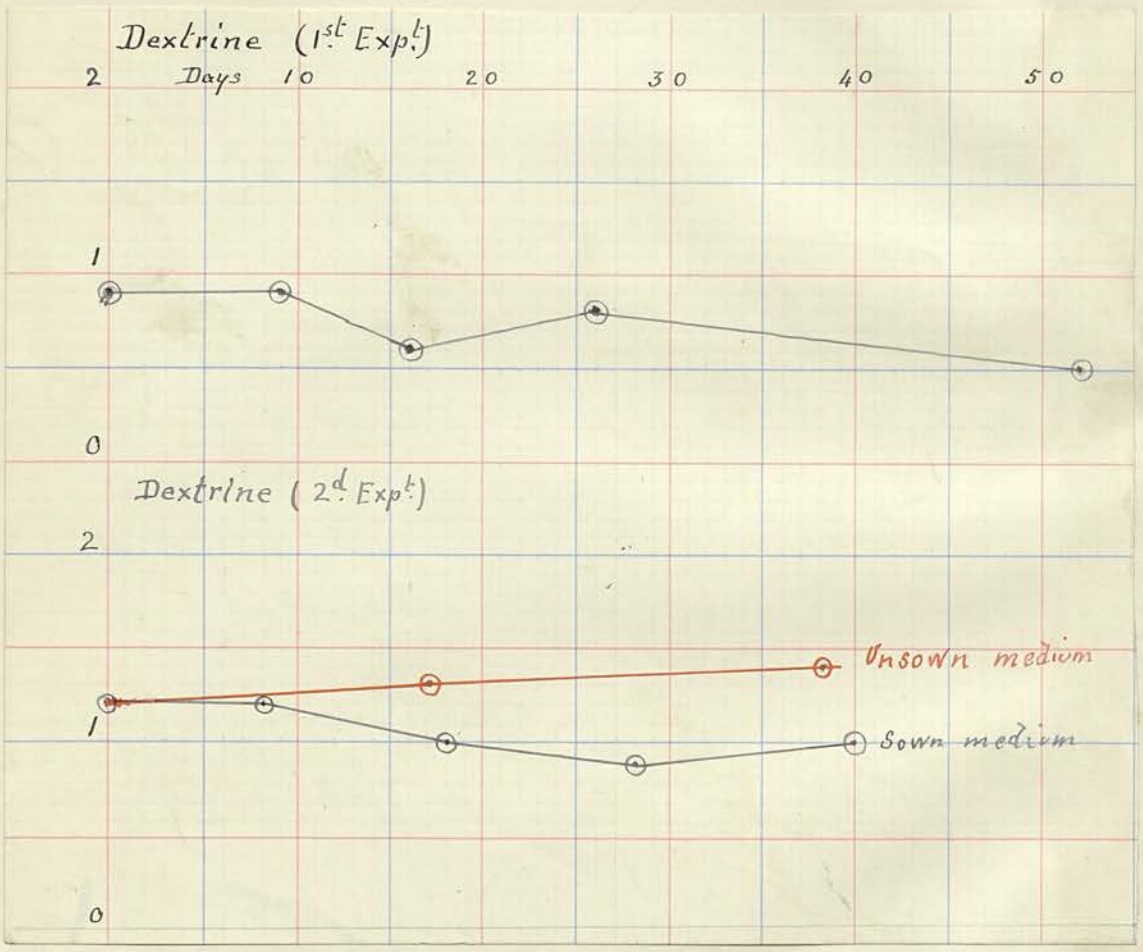
DULCITE. Sown with plague 13-5-07.

Date	Reaction.	Growth on Agar.	Growth in Broth.	Gas formation.
13-5-07	1.3	---	---	---
22-5-07	1.0	Pure; fair growth.	Surface growth, and abundant deposit.	None
1-6-07	1.1	Pure; good do.	Slight surface growth only.	None
11-6-07	0.6	Pure; thick do.	Slight surface growth, and along sides of tube. Abundant dept.	None
22-6-07	0.4	Pure; fair do.	Do. Do.	None.

DULCITE broth, not sown with anything.

Date.	Reaction.	
13-5-07	1.3	Colour same as ordinary broth medium.
1-6-07	1.6	
22-6-07	1.7	

D E X T R I N E



Good growth on Agar after 40 days in this medium.

FIRST GROUP

(Continued)

DEXTRINE. Prepared 5-2-07; sown with plague 21-2-07.

Date	Reaction.	Growth on Agar.	Growth in Broth.
21-2-07	0.9	----	----
2-3-07	0.9	----	----
9-3-07	0.6	Pure; abundant.	Abundant surface growth; thick deposit.
19-3-07	0.8	Pure	Do. Do.
15-4-07	0.5	Pure; good growth.	Very slight growth.

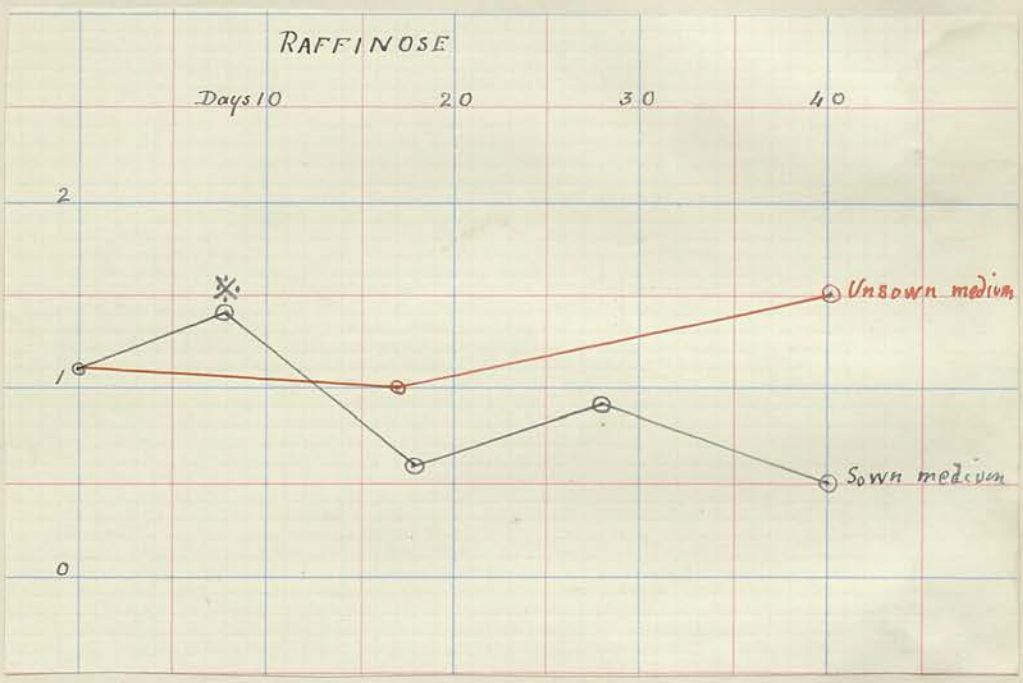
DEXTRINE. Sown with plague 13-5-07.

Date	Reaction.	Growth on Agar.	Growth in Broth.	Gas formation.
13-5-07	1.2	----	----	----
22-5-07	1.2	Pure; fair growth.	Abundant surface growth & deposit.	None.
1-6-07	1.0	Pure; good do.	Abundant surface & general growth.	None
11-6-07	0.9	Pure; uniform do.	Very good surface growth; abdt. deposit.	None
22-6-07	1.0	Pure; good do.	Some surface growth, abundant deposit.	None

DEXTRINE broth, not sown with anything.

Date.	Reaction.	
13-5-07	1.2	Colour same as ordinary medium.
1-6-07	1.3	
22-6-07	1.4	

R A F F I N O S E



Good growth on Agar after 40 days in this medium. The eighth day tube was contaminated and probably accounts for the high reading on that day.

F FIRST GROUP.

(Continued)

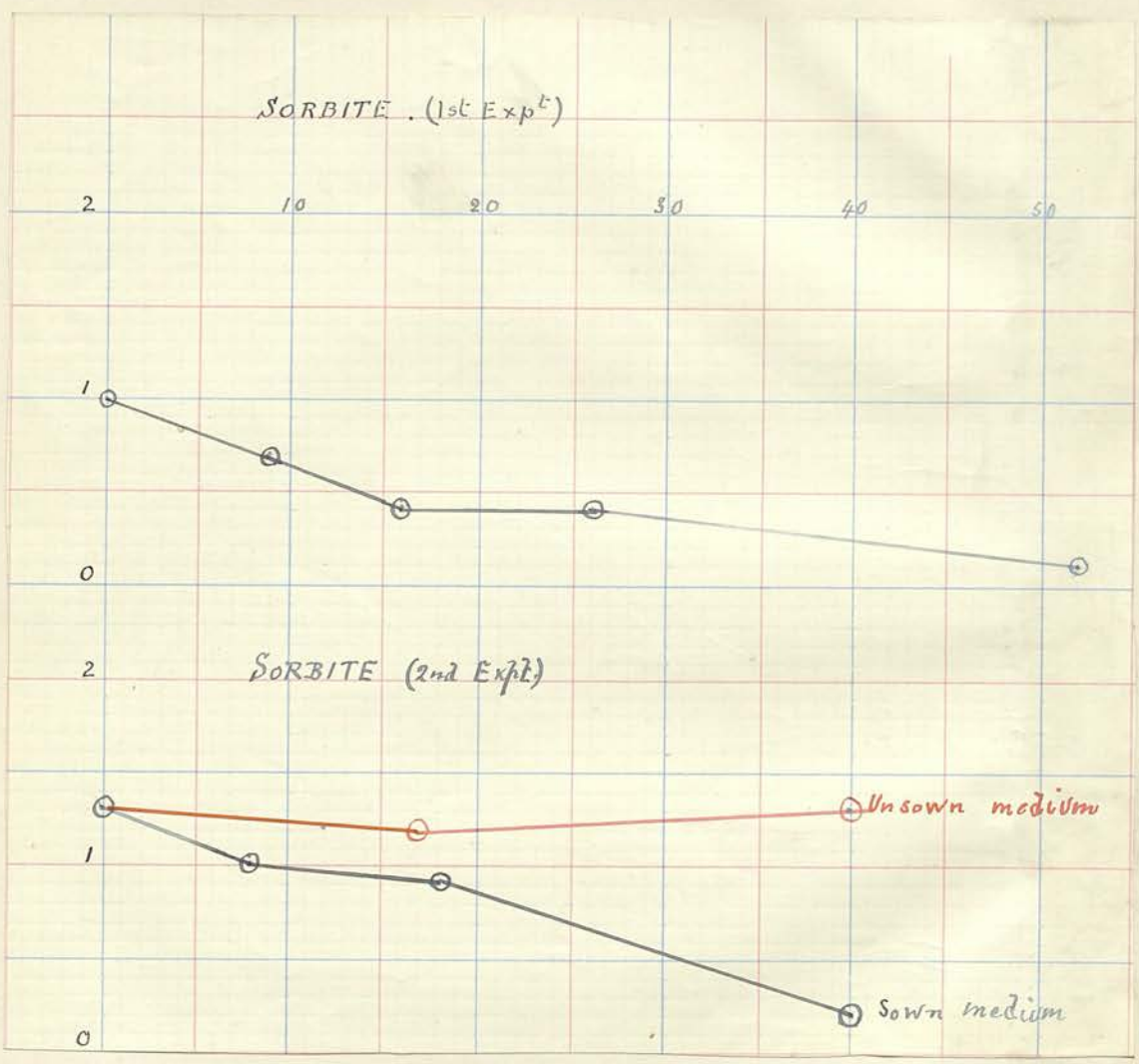
RAFFINOSE. Sown with plague 14-5-07.

Date	Reaction.	Growth on Agar.	Growth in Broth.	Gas formation.
13-5-07	1.1	----	----	----
22-5-07	1.4 (?)	Contaminated.	Abundant surface growth; abundant lumpy deposit. Query, pure ?	None.
1-6-07	0.6	Pure; good growth.	Abundant surface growth and much deposit.	None
11-6-07	0.9	Pure; uniform good growth.	Slight surface growth; abundant deposit.	None
22-6-07	0.5	Pure; good growth.	No surface growth but abundant deposit.	None.

RAFFINOSE broth, not sown with anything.

Date.	Reaction.	
13-5-07	1.1	Colour same as ordinary broth medium.
1-6-07	1.0	
22-6-07.	1.5	

S O R B I T E



Good growth on Agar after 40 days growth in this medium.

FIRST GROUP.

(Continued)

SORBITE. Prepared 5-2-07; sown with plague 21-2-07.

Date.	Reaction.	Growth on Agar.	Growth in Broth.
22-2-07	1.0	---	---
2-3-07	0.7	---	---
9-3-07	0.4	Pure; abundant.	Abundant surface growth and thick deposit.
19-3-07	0.4	Pure; good growth.	Scanty, on sides of tube only
15-4-07	0.1	Pure; good growth.	Slight growth.

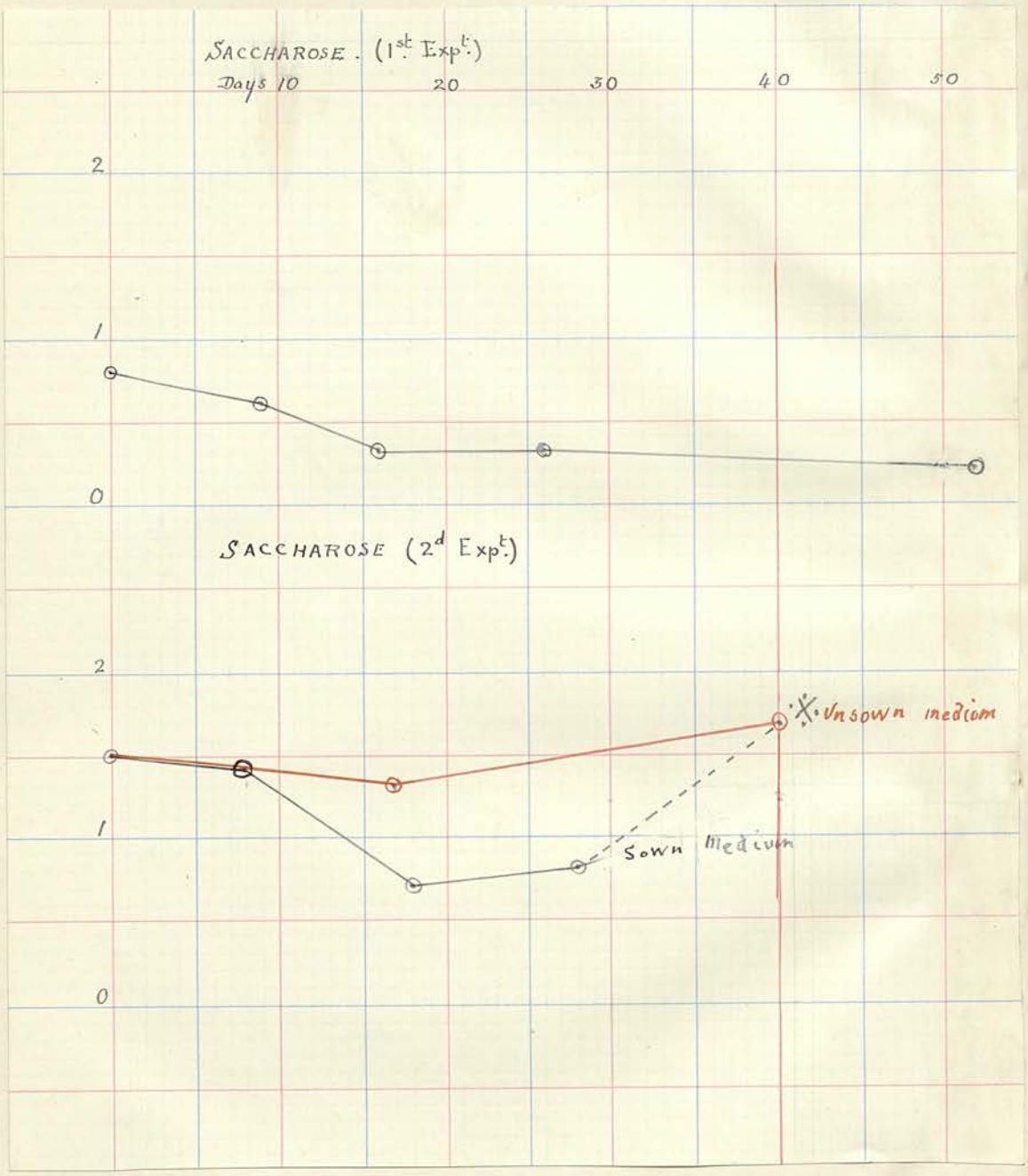
SORBITE. Sown with plague 14-5-07.

Date.	Reaction.	Growth on Agar.	Growth in Broth.	Gas formation
14-5-07	1.3	---	---	---
22-5-07	1.0	Pure; good growth.	Slight surface growth; fair lumpy deposit	None.
1-6-07	0.9	Pure; good growth.	Slight surface groth and along sides.	None
11-6-07	---	---	Slight surface grth. Abundant deposit.	None.
22-6-07	0.2	Pure; good growth.	Slight surface grth.; abundant deposit.	None.

SORBITE broth, not sown with anything.

Date.	Reaction.	
14-5-07	1.3	Colour same as orninary broth medium.
1-6-07	1.2	
22-6-07	1.3	

S A C C H A R O S E



Good growth on Agar after forty days in this medium.

X The last reading for the sown medium is probably wrong

FIRST GROUP.

(Continued)

SACCHAROSE. Prepared 5-2-07; sown with plague 22-2-07.

Date	Reaction.	Growth on Agar.	Growth in Broth.
22-2-07	0.8	---	---
2-3-07	0.6	---	---
9-3-07	0.3	Pure; abundant.	Abundant surface-growth, and thick deposit.
19-3-07	0.3	Pure; very good growth.	Similar to above, but less surface growth.
15-4-07	0.2	Pure; Do. Do.	No growth apparent, but heavy deposit.

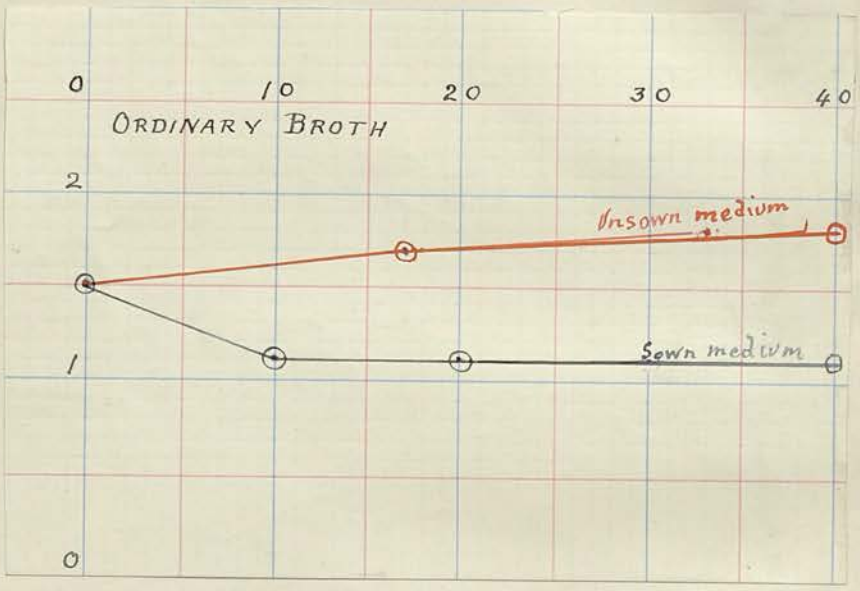
SACCHAROSE. Sown with plague 14-5-07.

Date.	Reaction.	Growth on Agar.	Growth in Broth.	Gas formation.
14-5-07	1.5	---	---	---
22-5-07	1.4	Pure; good growth.	Fair surface grth. considerable dept.	None.
1-6-07	0.7	Pure; good growth.	Abundant surface growth & deposit.	None.
11-6-07	0.8	Pure; good uniform growth.	Very scanty surface growth, fair grth. on sides.	None.
22-6-07	1.7 (?)	Pure; good growth.	Some surface grth. very abdt. deposit.	None.

SACCHAROSE broth, not sown with anything.

Date	Reaction.	
14-5-07	1.5	Colour same as ordinary broth medium.
1-6-07	1.3	
22-6-07	1.7	

O R D I N A R Y B R O T H



Good growth on Agar after forty days in this medium.

FIRST GROUP.

(Continued)

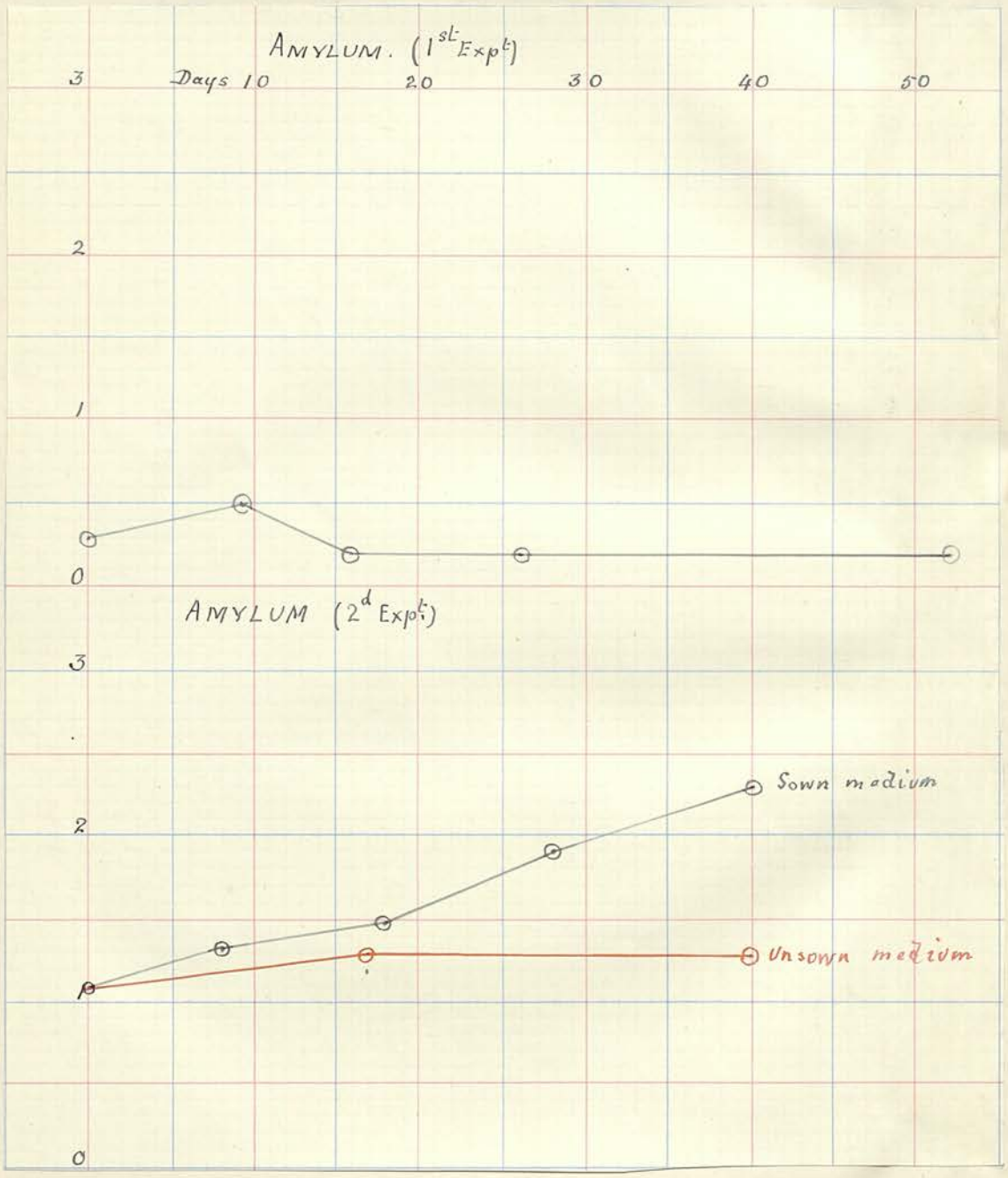
ORDINARY BROTH. Prepared 13-5-07; sown with plague 22-5-07.

Date.	Reaction.	Growth on Agar.	Growth in Broth.	Gas formation.
22-5-07	1.5	---	---	---
1-6-07	1.1	Pure; good growth.	Abundant surface growth & deposit.	None.
11-6-07	1.1	Pure; uniform growth.	Fair surface growth, abundant deposit, & some along sides	None.
22-6-07	1.1	Pure; good grth.	Some surface growth, Abundant deposit.	None

ORDINARY broth, not sown with anything.

Date.	Reaction.
15-5-07.	1.5
1-6-07	1.7
22-6-07	1.8

A M Y L U M



No growth on Agar after 19 days in this medium.

SECOND GROUP.

In which the B.pestis produces acid.

AMYLUM. Prepared 5-2-07; sown with plague 22-2-07.

Date	Reaction.	Growth on Agar.	Growth in Broth.
22-2-07.	0.3	---	---
2-3-07	0.5	Pure.	---
9-3-07	0.2	Pure; abundant.	Abundant surface growth and thick deposit.
19-3-07	0.2	Pure; good growth.	No surface growth, but fair deposit at bottom of tube.
15-4-07	0.2	Do. Do.	No growth apparent.

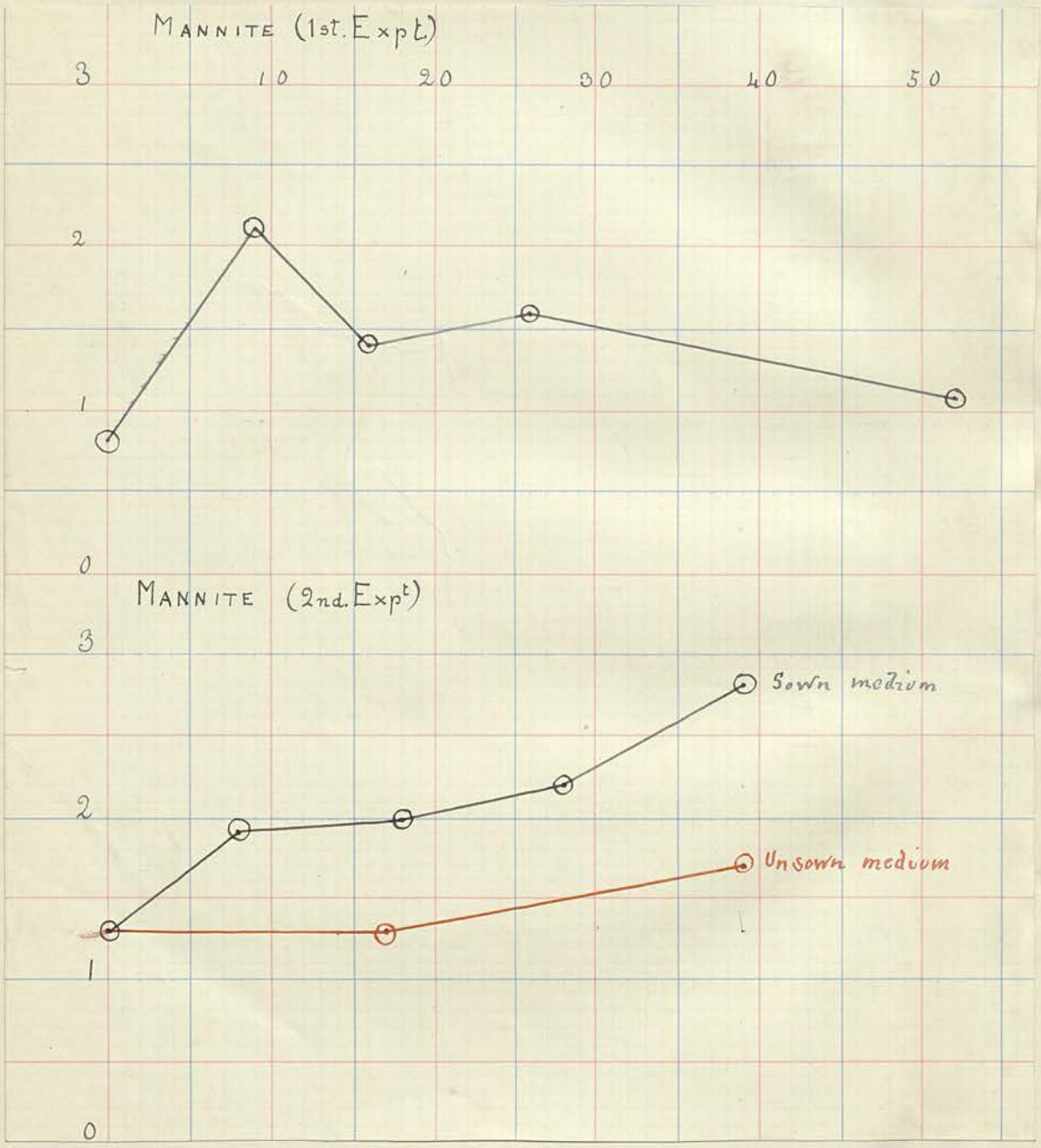
AMYLUM. Sown with plague 13-5-07.

Date	Reaction.	Growth on Agar.	Growth in Broth.	Gas formation.
13-5-07	1.1	---	---	---
22-5-07	1.3	Good growth, but contaminated.	No surface growth, but some along sides.	None.
1-6-07	1.5	None.	No surface growth, slight along sides.	None.
11-6-07	1.9.	None.	No surface growth, but scanty deposit.	None.
22-6-07	2.3	None.	Do. Do. Do.	None.

AMYLUM broth, not sown with anything.

Date	Reaction.	
13-5-07	1.1	Colour slightly paler than ordinary broth medium, and also slightly muddy.
1-6-07	1.3	
22-6-07	1.3	

M A N N I T E



No growth on Agar after 19 days in this medium.

SECOND GROUP.

(Continued)

MANNITE. Prepared 5-2-07; sown with Plague 22-2-07.

Date	Reaction.	Growth on Agar.	Growth in Broth.
22-2-07	0.8	---	---
2-3-07	2.1	Pure	---
9-3-07	1.4	Pure; numbers of large isolated colonies.	No surface growth, fair deposit at bottom of tube. Supernatant fluid clear.
19-3-07	1.6	Pure; growth very scanty.	No surface growth, but some on sides of tube. Deposit as above.
15-4-07	1.1	Pure; two or three minute colonies.	Slight growth only.

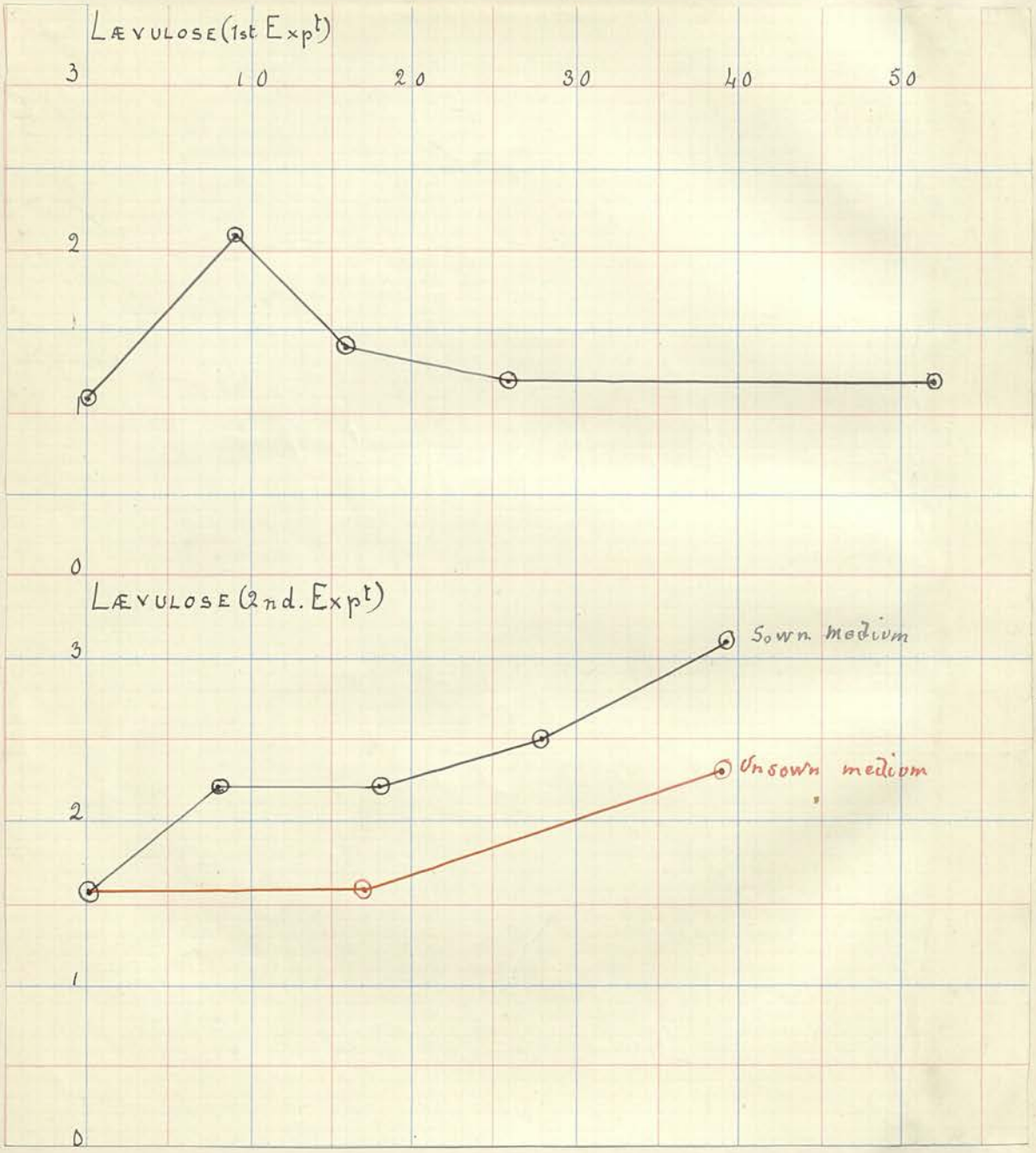
MANNITE. Sown with plague 13-5-07.

Date	Reaction.	Growth on Agar.	Growth in Broth.	Gas formation.
13-5-07	1.3	---	---	---
22-5-07	1.9	Pure; large discrete colonies.	No surface growth, Deposit fairly abundant.	None.
1-6-07	2.0	None.	No surface growth, scanty deposit.	None.
11-6-07	2.2	Pure; good grth.	Slight surface grth. and along sides. Slight deposit.	None.
22-6-07	2.8	None.	No surface growth, scanty deposit.	None

MANNITE broth, not sown with anything.

Date	Reaction.	
13-5-07	1.3	Same colour as ordinary broth medium.
1-6-07	1.3	
22-6-07	1.7	

L A E V U L O S E



No growth on Agar after 29 days in this medium.

SECOND GROUP.

(Continued)

LAEVULOSE. Prepared 5-2-07; sown with plague 21-2-07.

Date.	Reaction.	Growth on Agar.	Growth in Broth.
21-2-07	1.1	---	----
2-3-07	2.1	Pure, scanty.	----
9-3-07	1.4	Pure, very scanty.	No surface growth, but good along sides. Fair deposit.
19-3-07	1.2	None.	No surface growth, scanty along sides of tube.
15-4-07	1.2	None.	No growth apparent, but the deposit is considerable.

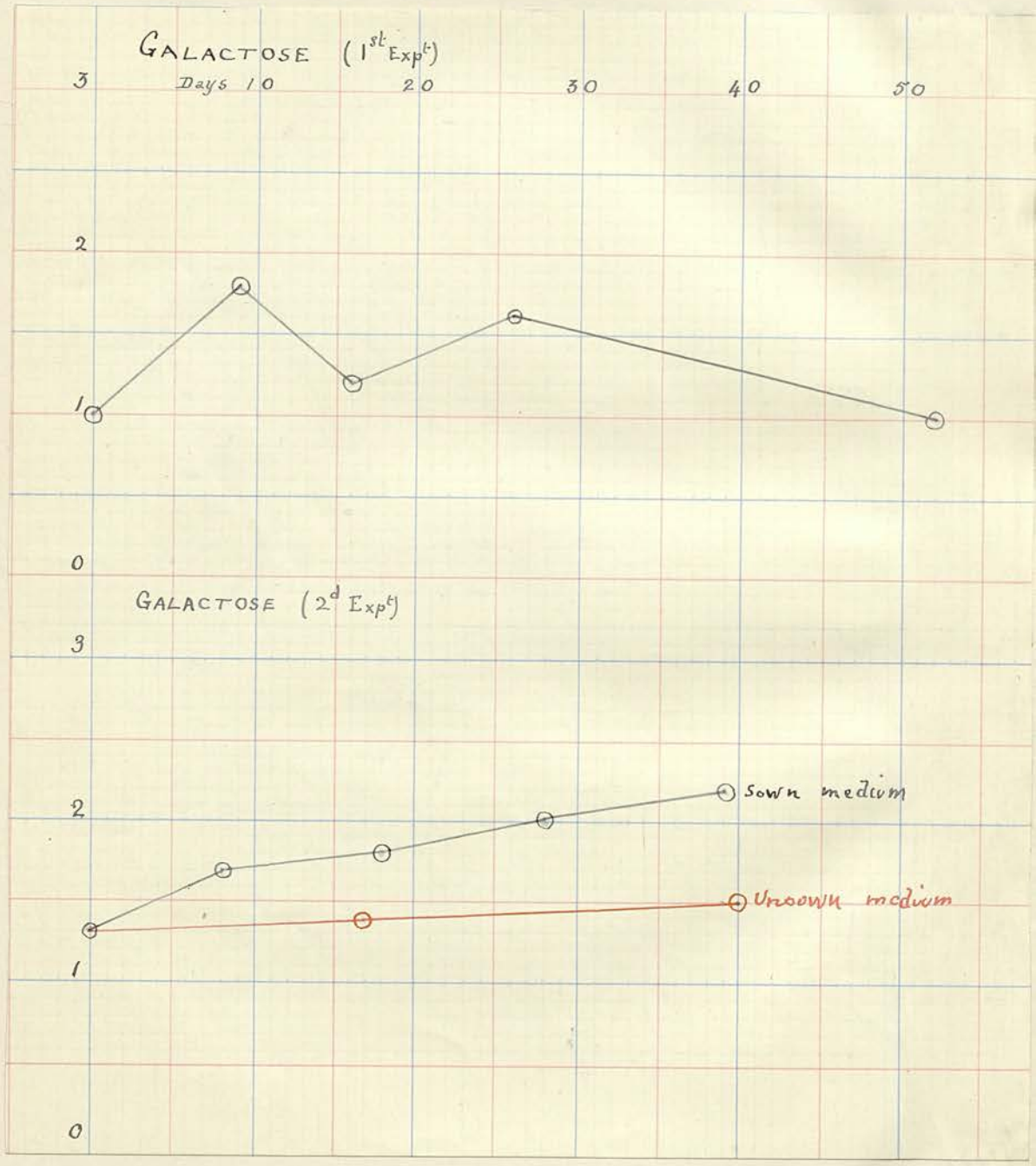
LAEVULOSE. Sown with plague 13-5-07.

Date.	Reaction.	Growth on Agar.	Growth in Broth.	Gas formation.
13-5-07	1.6	---	---	---
22-5-07	2.2	Pure; one or two very small cols.	No surface growth, abundant deposit.	None
1-6-07	2.2	Pure; many large scattered cols.	No surface growth, Slight along sides, Deposit scanty.	None.
11-6-07	2.5	None.	No surface or other growth. Fair deposit.	None.
22-6-07	3.1	None.	No surface growth. Abundant deposit.	None.

LAEVULOSE broth, not sown with anything.

Date	Reaction.	
13-5-07	1.6	
1-6-07	1.6	Colour darker than ordinary broth medium, which
22-6-07	2.3	may account for the higher readings of the titration, as the darkness of the fluid might affect the appearance of the pink reaction of the phenolphthalein.

G A L A C T O S E



No growth on Agar after 39 days in this medium.

SECOND GROUP.

(Continued)

GALACTOSE. Prepared 5-2-07; sown with plague 21-2-07.

Date	Reaction.	Growth on Agar.	Growth in Broth.
21-2-07	1.0	---	---
2-3-07	1.8	Pure.	---
9-3-07	1.2	Pure; only five Colonies.	No surface growth, but deposit with clear supernatant fluid.
19-3-07	1.6	None.	Do. Do. Do.
15-4-07	1.0	None	No growth apparent, thick deposit at bottom.

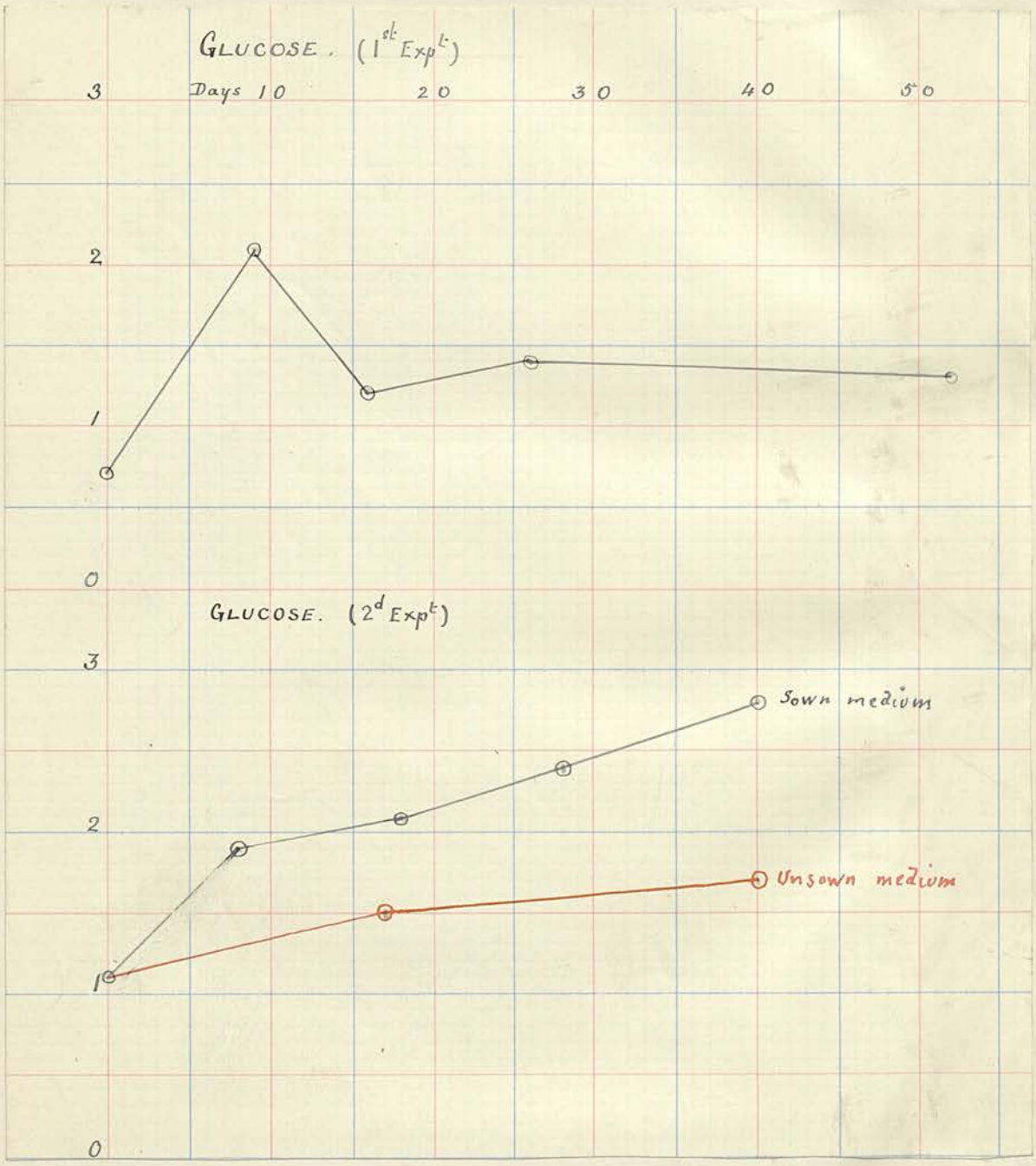
GALACTOSE. Sown with plague 13-5-07.

Date	Reaction	Growth on Agar	Growth in Broth.	Gas formation.
13-5-07	1.3	---	---	---
22-5-07	1.7	Pure; abundant grth.	Slight surface grth. Scanty deposit.	None.
1-6-07	1.8	Pure; good growth.	Slight surface growth, and along sides.	None.
11-6-07	2.0	Pure; 22 large colonies.	No surface growth. Slight growth on sides. Deposit slt.	None.
22-6-07	2.2	None.	No surface growth. Deposit scanty.	None.

GALACTOSE broth, not sown with anything.

Date.	Reaction.	
13-5-07	1.3	Same colour as ordinary broth medium.
1-6-07	1.4	
22-6-07	1.5	

G L U C O S E



No growth on Agar after 18 days in this medium.

SECOND GROUP.

(Continued)

GLUCOSE. Prepared 5-2-07; sown with plague 21-2-07.

Date	Reaction.	Growth on Agar.	Growth on Broth
21-2-07.	0.7	---	---
2-3-07	2.1	Pure; very scanty.	---
9-3-07	1.2	None.	No surface growth; slight along sides. Deposit considerable.
19-3-07	1.4	None.	No surface growth; slight along sides. Deposit at bottom.
15-4-07.	1.3	None.	No growth apparent. Deposit.

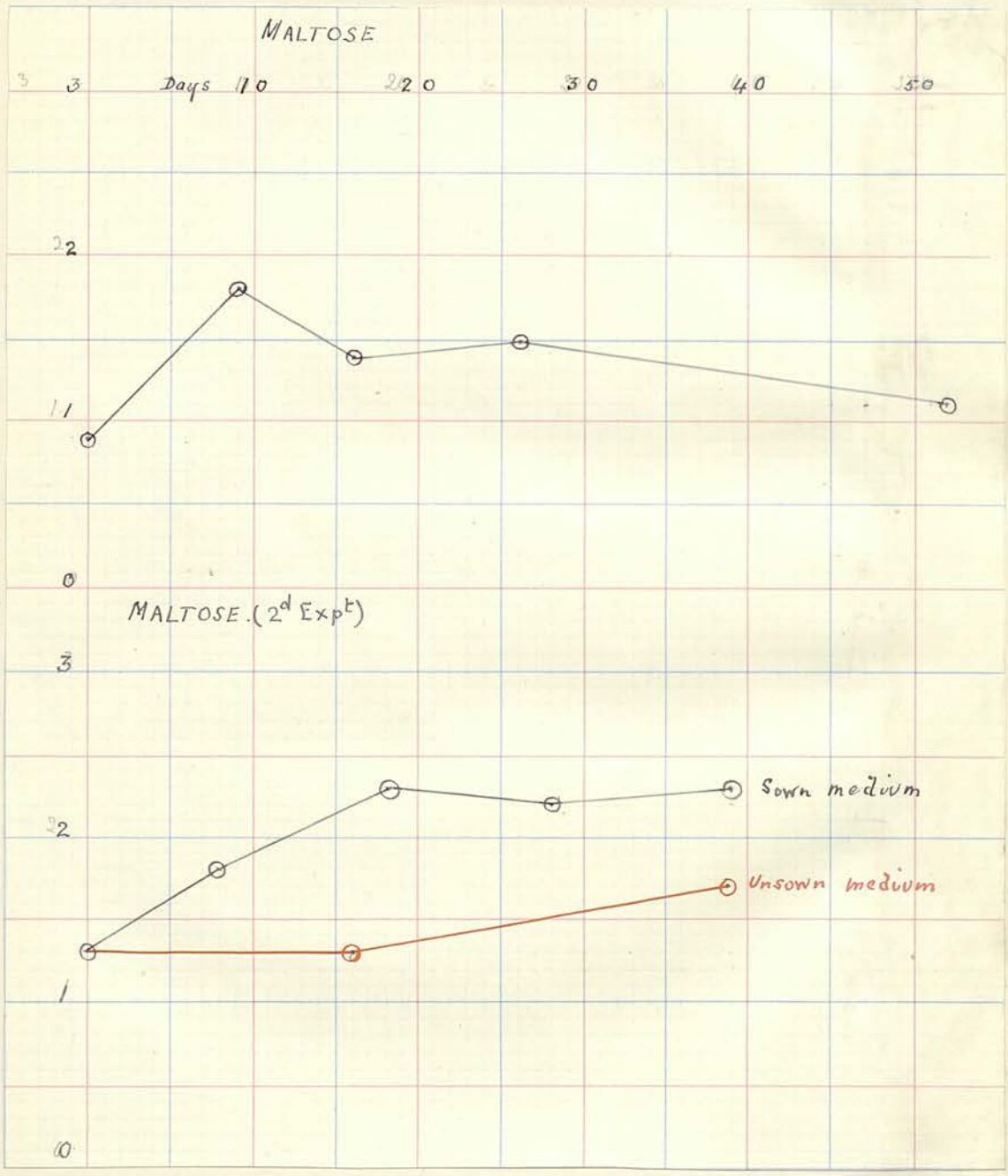
GLUCOSE. Sown with plague 14-5-07.

Date	Reaction.	Growth on Agar.	Growth in Broth.	Gas formation.
14-5-07	1.1	---	---	---
22-5-07	1.9	Pure; one or two colonies only.	No surface growth, slight on sides, abundant deposit.	None.
1-6-07	2.1	None.	No surface growth, Abundant deposit.	None.
11-6-07	2.4	None.	No surface growth. Slight along sides. Abundant deposit.	None.
22-6-07	2.8	None.	Slight surface grth. Abundant deposit.	None.

GLUCOSE broth, not sown with anything.

Date	Reaction.	
14-5-07	1.1	Same colour as ordinary broth medium.
1-6-07	1.5	
22-6-07	1.7	

M A L T O S E



No growth on Agar after 28 days in this medium.

SECOND GROUP

(Continued)

MALTOSE. Prepared 5-2-07; sown with plague 21-2-07.

Date	Reaction	Growth on Agar	Growth in Broth
21-2-07	0.9	---	---
2-3-07	1.8	Pure	---
9-3-07	1.4	Pure; 12 large colonies only	No surface growth, but some along sides. Some deposit.
19-3-07	1.5	None	No surface growth, scanty along sides of tube. Some deposit.
15-4-07	1.1	None	Slight surface growth and along sides. Some deposit.

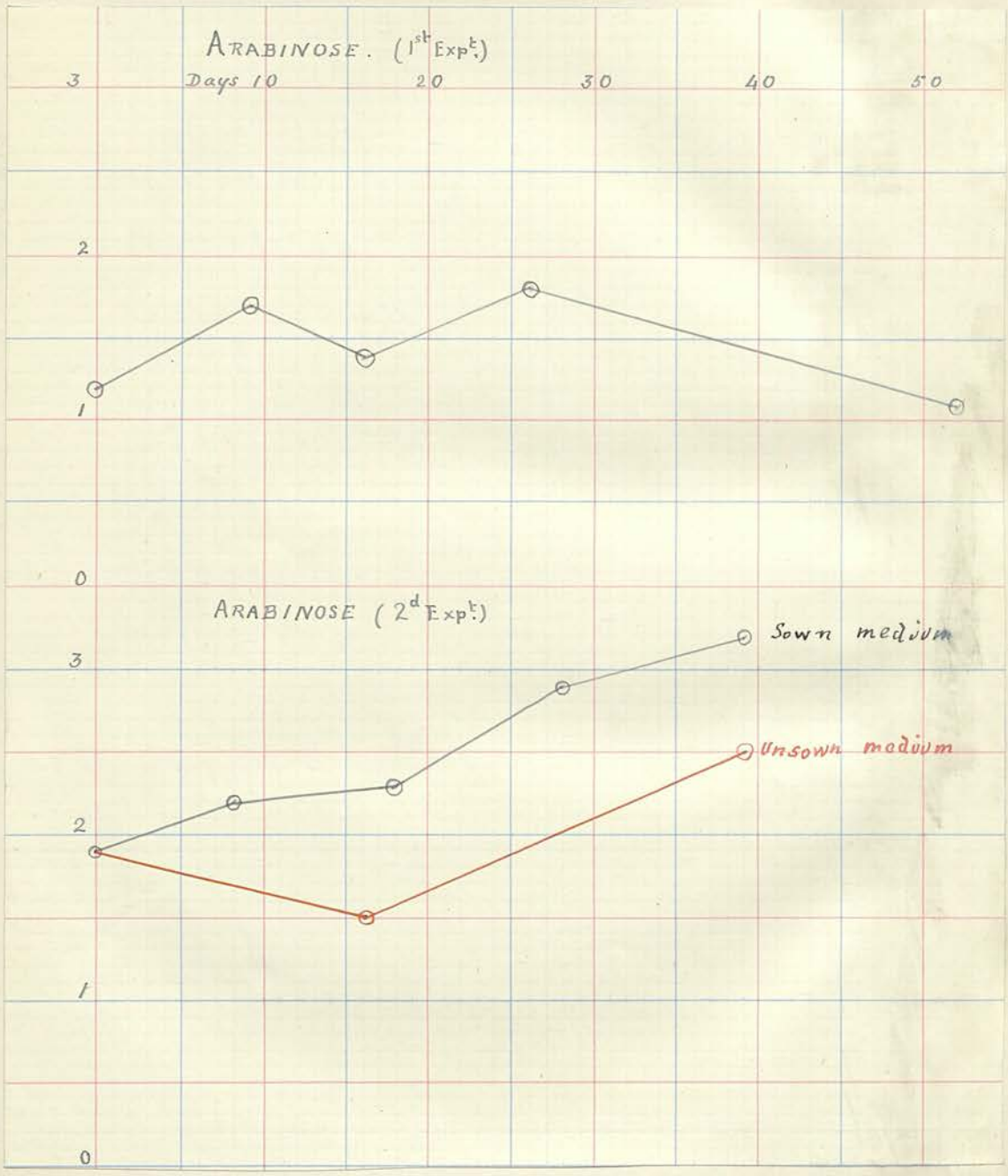
MALTOSE. Sown with plague 14-5-07.

Date	Reaction	Growth on Agar	Growth in Broth	Gas formation
14-5-07	1.3	---	---	---
22-5-07	1.8	Pure; good growth	No surface growth Slight gth.sides Some deposit	None
1-6-07	2.3	Pure; very few scattered cols.	No surface growth Some gth. sides. Slight deposit	None
11-6-07	2.2	None	No surface growth Some deposit	None
22-6-07	2.3	None	No surface growth Slight deposit.	None

MALTOSE broth not sown with anything.

Date	Reaction	
14-5-07	1.3	Same colour as ordinary broth medium
1-6-07	1.3	
22-6-07	1.7	

A R A B I N O S E



No growth on Agar after 28 days in this medium.

SECOND GROUP

(Continued)

ARABINOSE. Prepared 5-2-07; sown with plague 21-2-07

Date	Reaction	Growth on Agar	Growth in Broth
21-2-07	1.2	---	---
2-3-07	1.7	Pure	---
9-3-07	1.4	Pure; abundant	No surface growth. Some deposit Supernanant fluid clear
19-3-07	1.8	Pure; many large discrete colonies	Do. Do. Do.
15-4-07	1.1	None	Do. Do. Do.

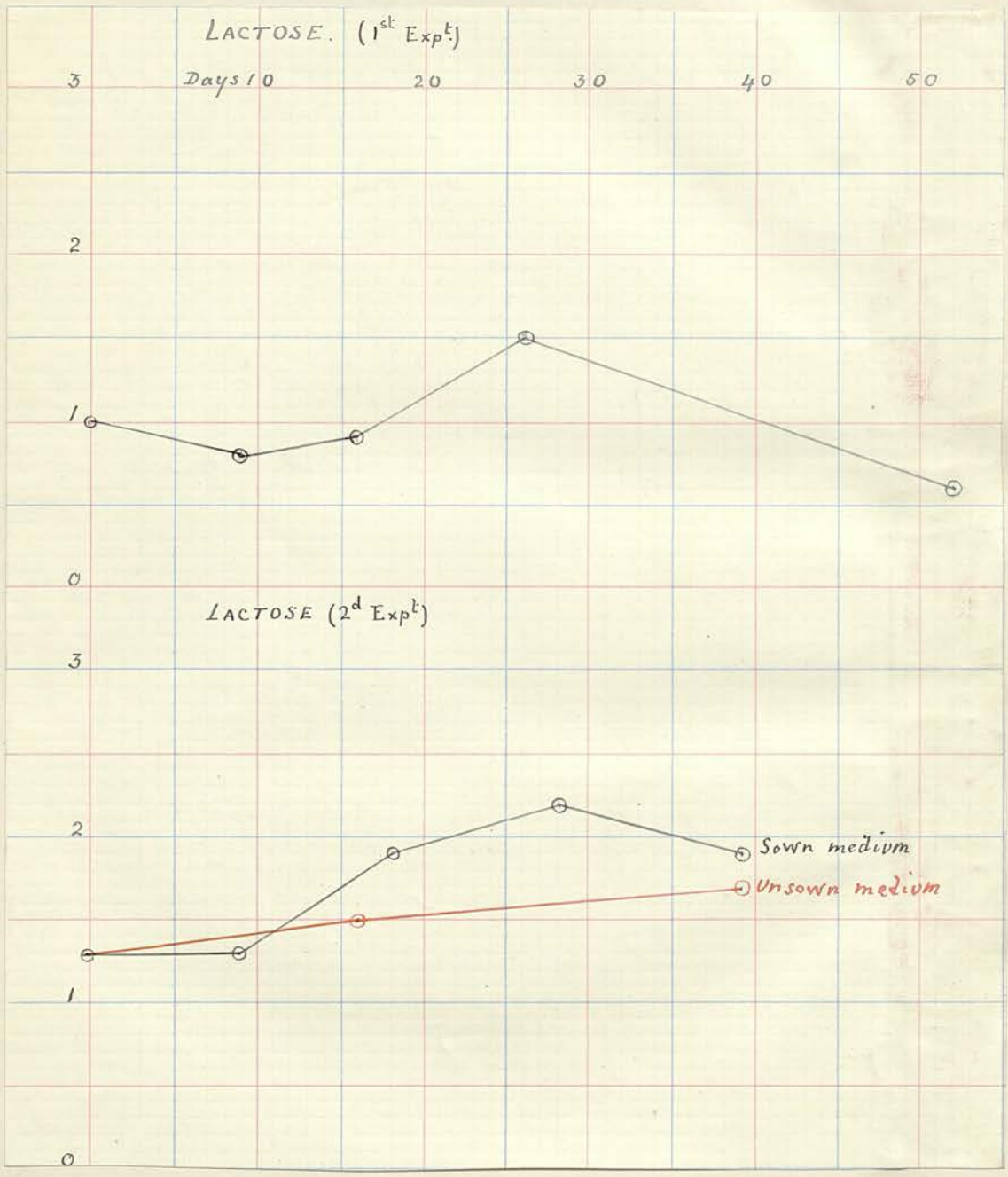
ARABINOSE. Sown with plague 14-5-07

Date	Reaction	Growth on Agar	Growth in Broth	Gas formation
14-5-07	1.9	---	---	---
22-5-07	2.2	Pure; good growth	No surface growth Scanty deposit	None
1-6-07	2.3	Pure; good growth	No surface growth Slight along sides	None
11-6-07	2.9	None	No surface growth Slight along sides Very slight dept.	None
22-6-07	3.2	None	No surface growth Very slight dept.	None

ARABINOSE broth not sown with anything.

Date	Reaction	
14-5-07	1.9	Colour darker than ordinary broth medium, which may account for the higher titration readings, as this might affect the appearance of the pink of the phenolphthalein reaction.
1-6-07	1.5	
22-6-07	2.5	

L A C T O S E



Bacillus not dead after 52 days in this medium.

SECOND GROUP

(Continued)

LACTOSE. Prepared 5-2-07; sown with plague 21-2-07.

Date	Reaction	Growth on Agar	Growth in Broth
21-2-07	1.0	---	---
2-3-07	0.8	Pure	---
9-3-07	0.9	Pure; abundant	Abundant surface growth and thick deposit
19-3-07	1.5	Pure; 12 discrete colonies only	Dp. Do. Do.
15-4-07	0.6	Pure; good growth	Good surface growth and abundant deposit.

LACTOSE. Sown with plague 14-5-07.

Date	Reaction	Growth on Agar	Growth in Broth	Gas formation
14-5-07	1.3	---	---	---
22-5-07	1.3	Pure; fair growth	Good surface grth.	None
1-6-07	1.9	Pure; good growth	Abundant surface growth & along sides Thick deposit	None
11-6-07	2.2	Pure; numerous scattered cols.	Abundant surface growth; also along sides. Thick deposit	None
22-6-07	1.9	Pure; good growth	Good surface growth and abundt. deposit	None

LACTOSE broth not sown with anything.

Date	Reaction	
14-5-07	1.3	Same colour as ordinary broth medium
1-6-07	1.5	
22-6-07	1.7	

ANIMAL EXPERIMENTS.

In view of the extent to which Haffkine's anti-plague vaccine is used throughout India, it seemed advisable to ascertain, if possible, whether a vaccine made by growing the plague bacillus in liquid media containing a percentage of any of the aforementioned substances, was more efficient as a preventive of plague than the vaccine as at present made. For this purpose it was necessary to use a large number of young guineapigs which had been bred in the laboratory, and whose history was therefore known. It has been our unfortunate experience in Bombay to find numbers of animal experiments fail, from the discovery that some of the animals used had already been exposed to epizootic plague, and had in consequence acquired a certain amount of immunity.

For the same reason the rats of Bombay could not be employed, nor could imported white rats be used, for we have found it impossible to get these exiles to breed under the tropical conditions of that place.

Undoubtedly in a country where plague epizootics do not occur, rats either wild or of the white variety, would be more suitable for the purpose than guineapigs.

The method of experiment was as follows. The guineapigs having been selected were carefully weighed, and arranged in order of weight. The range of variation in this experiment was from 400 to 220 grammes. The animals were then arranged so that each vaccine to be tested had some of the heavier and some of the lighter ones alloted to it.

In this way the various groups were equalised as to average weight. Each group was then treated with the particular vaccine which it was desired to test, each animal receiving 2.5 c.c. subcutaneously.

Ten days afterwards all the animals were injected subcutaneously with 0.25 c.c. of various dilutions of the same virulent culture of plague.

The method of making these dilutions, originally devised by Wright, has been fully described by Liston who modified the technique so as to make it applicable to work with the B.pestis: vide the Annual Report of the Plague Research Laboratory, Bombay, for the year 1905, p.15-16, where an illustrated account will be found. The dilutions are made immediately before injection and the cultures made at the same time enable one to estimate approximately the number of germs injected into each animal.

Owing to the number of the different substances under trial, a very large series of comparable experimental animals had to be used.

As a consequence the number available for any one vaccine was small. From a study of the tables however, and taking the grand totals only, into account a good idea of the comparative efficiency of the vaccines of the two groups may be got, and the way is cleared for future investigation.

As will be seen from the tables on

pages 48 to 51, the dilutions used were 1-in-100, 1-in-1000, 1-in-10,000, and 1-in-100,000 of a 48-hours-old culture of the plague germ in rat broth. For each of these dilutions three control animals were used, and originally I had intended to use the same number for each of the corresponding dilutions injected into the vaccinated guineapigs. Owing to various causes it was found impracticable to do this, hence the different numbers shown opposite the treated animals.

On the morning of injection, and daily thereafter, the guineapigs were weighed, as we have found by experience that this gives quite as satisfactory a record of the animal's condition from day to day, as the more tedious method of taking the temperature in the rectum. From this daily record the tables have been compiled, showing the number of guineapigs remaining alive and the number dying during each week.

The following table summarises the results which have been calculated on the total number of guineapigs vaccinated with ~~each~~ the chemical bodies in which the B.pestis produces respectively an alkaline or an acid reaction, compared with control animals which had not been vaccinated.

Number of animals used.	Substance with which animal was vaccinated	Percentage Mortality		
		After 7 days	After 14 days	After 21 days
12	Not vaccinated	Nil	25.1	41.6
46	Group No.I	Nil	19.5	34.7
56	Group No.II	Nil	17.8	44.6

From a consideration of these figures it is clear:-

(1) That the vaccinated groups have a distinct advantage over the non-vaccinated at the end of 14 days from the date of injection with plague.

(2) That in the case of those vaccinated with the vaccine prepared from the substances in which the plague germ produces acid, this advantage has disappeared adter 21 days; the non-vaccinated and the vaccinated being practically the same as regards the percentage mortality.

(3) That on the other hand those vaccinated with the vaccine prepared from the substances in which the plague germ produces alkali, show a considerable degree of immunity at the end of three weeks, as compared with the non-vaccinated animals; the amount of protection being very similar to that produced by the anti-plague vaccine prepared by growing the B.pestis in the ordinary laboratory medium.

From these considerations I think we may conclude that qua the vaccines there is no advantage to be gained by growing the Bacillus

pestis in media to which any of these chemical bodies have been added, and that we must look in other directions for means of improving the manufacture of the anti-plague vaccine.

Table showing the result of animal experiments after weekly intervals subsequent to inoculation.

Media	Dilutions	After 7 days		After 14 days		After 21 days	
		Alive	Dead	Alive	Dead	Alive	Dead
CONTROLS	100	3	0	1	2	1	2
	1000	3	0	2	1	2	1
	10,000	3	0	3	0	1	2
	100,000	3	0	3	0	3	0
	Total	12	0	9	3	7	5
Mortality per cent		Nil		25.0		41.6	
Group I, in which alkali is produced.							
INULIN	100	2	0	2	0	2	0
	1000	2	0	1	1	1	1
	10,000	2	0	2	0	1	1
	100,000	2	0	2	0	2	0
	Total	8	0	7	1	6	2
DULCITE	100	2	0	2	0	0	2
	1000	2	0	1	1	1	1
	10,000	2	0	2	0	2	0
	100,000	2	0	2	0	1	1
	Total	8	0	7	1	4	4
DEXTRINE	100	2	0	1	1	0	2
	1000	2	0	0	2	0	2
	10,000	2	0	2	0	2	0
	100,000	2	0	2	0	2	0
	Total	8	0	5	3	4	4
RAFFINOSE	100	2	0	1	1	1	1
	1000	2	0	2	0	2	0
	10,000	2	0	1	1	1	1
	100,000	2	0	1	1	1	1
	Total	8	0	5	3	5	3

Animal Experiments; Group I, continued.

Media	Dilutions	After 7 days		After 14 days		After 21 days	
		Alive	Dead	Alive	Dead	Alive	Dead
SORBITE	100	2	0	2	0	1	1
	1000	2	0	2	0	2	0
	10,000	2	0	2	0	2	0
	100,000	2	0	2	0	2	0
	Total	8	0	8	0	7	1
SACCHAROSE	100	2	0	1	1	1	1
	1000	---	---	---	---	---	---
	10,000	2	0	2	0	2	0
	100,000	2	0	2	0	1	1
	Total	6	0	5	1	4	2
Grand total		46	0	37	9	30	16
Mortality per cent		Nil		19.5		34,7	

Animal Experiments, continued; Group II.

Media	Dilutions	After 7 days		After 14 days		After 21 days	
		Alive	Dead	Alive	Dead	Alive	Dead
Amylum	100	3	0	3	0	2	1
	1000	3	0	3	0	1	2
	10,000	3	0	2	1	2	1
	100,000	1	0	0	1	0	1
	Total	10	0	8	2	5	5
MANNITE	100	2	0	1	1	1	1
	1000	2	0	2	0	1	1
	10,000	2	0	2	0	0	2
	100,000	2	0	2	0	2	0
	Total	8	0	7	1	4	4
LAEVULOSE	100	2	0	1	1	1	1
	1000	2	0	2	0	1	1
	10,000	2	0	1	1	1	1
	100,000	2	0	2	0	1	1
	Total	8	0	6	2	4	4
GALACTOSE	100	2	0	1	1	1	1
	1000	2	0	1	1	1	1
	10,000	2	0	2	0	0	2
	100,000	2	0	2	0	1	1
	Total	8	0	6	2	3	5
GLUCOSE	100	1	0	0	1	0	1
	1000	2	0	2	0	2	0
	10,000	2	0	2	0	2	0
	100,000	2	0	2	0	2	0
	Total	7	0	6	1	6	1

Animal Experiments, continued; Group II.

Media	Dilutions	After 7 days		After 14 days		After 21 days	
		Alive	Dead	Alive	Dead	Alive	Dead
MALTOSE	100	2	0	2	0	2	0
	1000	--	--	--	--	--	--
	10,000	1	0	0	1	0	1
	100,000	2	0	2	0	1	1
	Total	5	0	4	1	3	2
ARABINOSE	100	2	0	2	0	1	1
	1000	2	0	1	1	1	1
	10,000	2	0	2	0	1	1
	100,000	1	0	1	0	1	0
	Total	7	0	6	1	4	3
LACTOSE	100	--	--	--	--	--	--
	1000	1	0	1	0	0	1
	10,000	2	0	2	0	2	0
	100,000	--	--	--	--	--	--
	Total	3	0	3	0	2	1
Grand total		56	0	46	10	31	25
Mortality per cent.		Nil		17.8		44.6	